

Exploring the role of miRNAs in the diagnosis of MODY3

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Background/aim: MODY3 associated with HNF1A is the most common form of MODY and is clinically misdiagnosed as type 1 diabetes due to similar clinical symptoms. This study aimed to analyze the role of HNF1A-regulated miRNAs as a biomarker in the diagnosis of MODY3.

Materials and methods: MIN6 cells were transfected with the HNF1A cDNA expression vector for overexpression or with siRNA specific to HNF1A to silence its expression. The HNF1A-regulated miRNAs were determined by RNA-Seq of the total RNA extract. Expressions of the candidate miRNAs in blood samples of MODY3, type 1 diabetes, and type 2 diabetes patients and in healthy subjects were compared statistically by Mann-Whitney U tests.

Results: This study revealed the presence of 238 known HNF1A-regulated miRNAs in MIN6 cells. miR-129-1-3p, miR-200b-3p, and miR-378a-5p were selected as candidate miRNAs. The expression level of miR-378a-5p significantly decreased in type 2 diabetes and MODY3 patients, while miR-200b-3p expression was significantly decreased only in MODY3 patients.

Conclusion: Although further studies with larger numbers of patients are required, this study demonstrated that the expression levels of miR-200b-3p and miR-378a-5p decrease in MODY3 patients and suggests that miR-200b-3p is an especially strong candidate to use clinically for selecting suspected MODY3 patients.

Key words: Biomarker, diabetes, miRNA, MODY3, miR-200, miR-378

1. Introduction

Maturity-onset diabetes of the young (MODY) is an inherited early-onset nonautoimmune form of diabetes. MODY is a clinically heterogeneous disease with 13 different phenotypes regarding the onset of the disease, the level of hyperglycemia, and the response to the treatment. Although it is classified as a monogenic disease with autosomal dominant inheritance, it is a genetically heterogeneous disease that is currently associated with 13 different genes. Among the 13 different genetic types, MODY3 associated with the hepatocyte nuclear factor transcription factor 1 alpha (HNF1A) gene is the most common form of MODY in adults with 30%–60% prevalence among MODYs. Approximately 70% of MODY3 patients develop this disease before age of 25. It is

associated with functional deficiency of HNF1A. HNF1A is a protein with 631 amino acids containing amino terminal dimerization, pseudo-POU and homeodomain-containing DNA binding, and carboxyl terminal transactivation domains. The HNF1A gene is localized on chromosome 12q24.31, spanning a region of 120 kb with 10 exons (1). It is expressed in different tissues including pancreatic islets, kidneys, intestines, and the liver. The HNF1A gene is expressed late in embryonic development of the pancreas, where it is confined to developing islet cells, and in the mature pancreas (2–4). Studies with HNF1A-knockout transgenic mice have demonstrated reduced beta cell numbers when the HNF1A gene is turned off (5) and the development of impaired glucose-induced insulin secretion associated with diabetes (6,7).

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Micro RNAs (miRNAs) are ~22-nucleotide noncoding single-stranded ribonucleic acids that can play important roles in posttranscriptional regulation of gene expression. It is estimated that there are more than 2000 miRNAs in the human genome (www.mirbase.org) and these miRNAs regulate 30% of the human transcripts (8,9). Recent studies have revealed that miRNAs have important roles in pancreatic development and beta cell differentiation as well as the development of diabetes, diabetic complications, insulin expression, insulin secretion, and insulin action (10–12). It has been shown that the serum miRNA expression profile differs significantly in type 2 diabetes patients with diabetic retinopathy (13). It was demonstrated that when HNF1A is mutated to have the common human Pro29finsC-HNF1A frameshift mutation, miR-103 and miR-224 were overexpressed (14).

We report here the identification of HNF1A-regulated miRNAs, which may play important roles in the pathogenesis of MODY3, and we explore the use of candidate miRNAs as a biomarker for the diagnosis of MODY3.

2. Materials and methods

2.1. MIN6 cell culture

Pancreatic β -cell line mouse insulinoma 6 (MIN6) cells derived from mouse pancreas (AddexBio, San Diego, CA, USA) were cultured in complete Dulbecco's modified Eagle's medium (DMEM) containing 25 mM glucose (high glucose), 15% (v/v) fetal bovine serum, 1% penicillin/streptomycin, 2 mM glutamine, and 100 mM β -mercaptoethanol at 37 °C in 5% CO₂.

2.2. Transfection of MIN6 cells for HNF1A overexpression
MIN6 cells were cultured in 6-well plates and transfected with 1 μ g of human HNF1A expression vector to overexpress the HNF1A gene using Xtremegene Gene HP transfection reagent (Ref.: 06366236001, Roche Life Science, USA) as described in the manufacturer's protocol for 48 h. The expression vector expressing the wild-type human HNF1A gene was kindly provided by Dr Eiji Awasaki from the University of Nagasaki, Japan. Transfection studies were performed in triplicates.

2.3. Transfecting MIN6 cells for HNF1A silencing
MIN6 cells cultured in 6-well plates were transfected with Lipofectamine RNAimax Reagent HNF1A-specific siRNA purchased to silence the expression of the HNF1A gene (Cat. No.: 13778150, Thermo Fisher Scientific, USA). Silencing experiments were performed in triplicates.

2.4. Total RNA isolation

Total RNA was isolated from the HNF1A expression vector and HNF1A siRNA transfected cells using the mirVana miRNA isolation kit (Ambion Thermo Fisher Scientific, USA) according to the manufacturer's protocol.

2.5. Confirming the overexpression and silencing of HNF1A

In order to confirm the overexpression and silencing of HNF1A in MIN6 cells, HNF1A gene expression was assessed using quantitative real-time PCR (qRT-PCR) based on the TaqMan probe system (Thermo Fisher Scientific, USA). For qRT-PCR, cDNA was synthesized from total RNA samples isolated from transfected cells by using a Transcriptor First Strand cDNA Synthesis Kit (Roche, Germany).

qRT-PCR reaction was performed using the FastStart Essential DNA Probes Master and Real-Time Ready Custom Single Assay (Roche Life Science, USA), while the human HNF1A assay (ID: 112176) for HNF1A plasmid and mouse HNF1A assay (ID: 316865) for internal expression of HNF1A were used. Mouse GAPDH assays (ID: 307884) were utilized for normalization.

The data were normalized to the endogenously expressed GAPDH and relative expression was calculated by $\Delta\Delta C_t$ method (15). qRT-PCR experiments were run in duplicate and P-values were calculated by Mann-Whitney test. $P < 0.05$ was considered statistically significant.

2.6. Western blot analysis

To validate the HNF1A overexpression and silencing in protein levels, western blot analysis was carried out using the Bolt Welcome Pack + iBlot 2 System (Invitrogen, USA). To detect HNF1A protein produced in transfected cells, rabbit polyclonal HNF1A antibody was used (Santa Cruz, USA). As an internal control, monoclonal β -actin antibody was used (Cell Signaling, USA). For detection, the Opti-4CN Detection Kit with goat antirabbit antibody was used (Biorad, USA).

Western blot analysis was also performed for the MIN6 cells that were not transfected as a control. ImageJ software was used to quantitate the band intensity in the blot. Each band was measured twice.

2.7. Next-generation sequencing of total RNA samples

After verifying the overexpression and silencing of HNF1A in MIN6 cells, total RNA samples isolated from transfected cells were sent to BGI, South Korea, for miRNA sequencing with the use of Illumina TruSeq technology.

2.8. In silico prediction of targets genes

The possible targets of the differentially expressed miRNAs were predicted by using the miRanda (www.microrna.org/microrna/home.do) and TargetScan (www.targetscan.org) databases on 18 August 2017. Then the targets were classified according to signaling pathways by using the Panther classification system (<http://pantherdb.org>), accessed on 22 August 2017.

2.9. Validation of miR-129-1-3p, miR-200b-3p, and miR-378a-5p expression

In order to validate the miR-129-1-3p, miR-200b-3p, and miR-378a-5p as HNF1A targets, their expression levels were analyzed by qRT-PCR in HNF1A overexpressed and

silenced MIN6 cells as described above. TaqMan probes were purchased from Thermo Fisher Scientific: hsa-miR-129-1-3p (AAGCCCUUACCCCAAAAAGUAU), hsa-miR-200b-3p (UAAUACUGCCUGGUAUUGAUGA), and hsa-miR-378a-5p (CUCCUGACUCCAGGUCCUGUGU).

2.10. Analysis of the role of miR-129-1-3p, miR-200b-3p, and miR-378a-5p as biomarkers for the diagnosis of MODY3 patients

The expression levels of miR-129-1-3p, miR-200b-3p, and miR-378a-5p were compared in blood samples of the study populations, which consisted of MODY3 patients (n = 4), type 1 diabetes (n = 5), and type 2 diabetes (n = 5) patients and healthy controls (n = 3), which were analyzed using miRNA-specific TaqMan probes (Thermo Fisher Scientific, USA) with qRT-PCR as described above and compared statistically using Mann-Whitney U tests. Blood samples were collected in Paxgene Blood RNA Tubes (Cat No.: 762165, BD Biosciences, Switzerland). MODY3 patients, referred to our laboratory by the Turkish Diabetes Foundation, were previously determined by NGS analysis of the HNF1A gene by us (unpublished data). Blood samples of type 1 and type 2 diabetes patients were also provided by the Turkish Diabetes Foundation. Control blood samples were obtained from individuals without a familial diabetes history who were nonobese and younger than 40 years old. The control subjects were identified clinically as nondiabetic and without major clinical risk factors for diabetes. Ethical approval for this study was obtained from the ethical committee of İstanbul Arel University.

3. Results

3.1. Overexpression and silencing of the HNF1A gene in MIN6 cells

Overexpression and silencing of the HNF1A gene in transfected MIN6 cells were verified by qRT-PCR. As a control, MIN6 cells not transfected were used to compare the overexpression and silencing of the HNF1A gene.

Both human and mouse HNF1A levels were significantly increased in the overexpression group but decreased in the silencing group as compared with the control group (Figure 1). The significant increase in mouse HNF1A expression level is possibly due to the transcription factor activity of HNF1A, which activates its own expression in a positive feedback mechanism (16).

3.2. Western blot analysis of HNF1A in overexpressed and silenced MIN6 cells

HNF1A overexpression and silencing in transfected MIN6 cells were also validated by western blot for protein levels. HNF1A protein levels (67,325 kDa) were measured by normalizing them to the housekeeping protein β -actin (Figure 2). Protein densities on the membrane were measured using ImageJ software to have quantitative

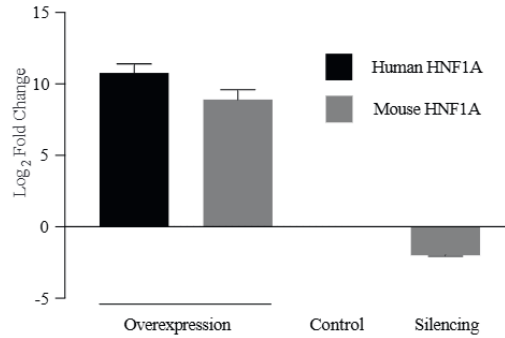


Figure 1. Validation of the overexpression and siRNA induced silencing of HNF1A gene in transfected MIN6 cells by qRT-PCR. Control represents the expression in MIN6 cells not transfected. *: Statistically significant.



Figure 2. Western blot analysis of HNF1A overexpression and siRNA induced silencing in MIN6 cells. O, Overexpression; S, silencing; C, control.

information about the expression levels of HNF1A. The β -actin bands were also measured to normalize the HNF1A (Figure 3). HNF1A was almost 2.75 times more expressed and 75% less expressed in overexpressed and silenced cells, respectively, compared to the control.

3.3. Identification of HNF1A-regulated miRNAs

miRNA sequencing of the RNA extracts from HNF1A overexpressed or silenced cells revealed that 238 known

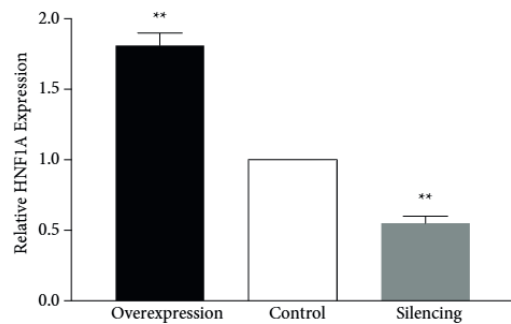


Figure 3. Densitometric analysis of western blots of HNF1A overexpression and siRNA induced silencing in MIN6 cells. O, overexpression; S, silencing; C, control.

miRNAs in the miRBase database that were predicted by MIREAP and Mirdeep software have significant differential expression (Supplementary Table S1).

In order to have more valid data, additional filtering steps were applied to the miRNA sequencing analysis for the known miRNAs. miRNAs having $\log_2$ -fold change lower than 1 were eliminated. After that, miRNAs that had the most significant ($P < 0.05$) differential expression were selected. miRNAs that passed through these filtering steps are shown in Table 1.

In order to determine the potential targets of differentially expressed miRNAs, database analysis was performed using the miRanda and TargetScan target

prediction tools and classification of the targets with Panther algorithms. In silico prediction of target signaling pathways revealed that the insulin/IGF pathway, protein kinase B signaling pathway, Wnt signaling pathway, and glycolysis pathway are among the targets of miR-129-1-3p, miR-200b-3p, and miR-378a-5p.

3.4. Analysis of the role of miR-129-1-3p, miR-200b-3p, and miR-378a-5p as biomarkers for the diagnosis of MODY3 patients

miR-129-1-3p, miR-200b-3p, and miR-378a-5p were selected as candidate miRNAs to explore their roles as biomarkers to diagnose MODY3.

Table 1. Fold change and P-values of known miRNAs having the most significant differential expression. P-values smaller than $1e-350$ are shown as 0.

Name	$\log_2$ fold change (overexpression/silencing)	P-value
miR-129-1-3p	2.63	0
miR-129-2-3p	2.87	0
Let-7b-3p	2.46	$7.6e-15$
miR-378a-5p	2.24	$6.7e-30$
miR-3072-3p	2.41	$7.74e-37$
miR-8112	1.91	$4.48e-35$
miR-200b-3p	1.02	$7.34e-30$
miR-324-5p	2.26	$2.12e-26$
miR-296-3p	2.50	$7.33e-6$
miR-1983	2.01	$2.73e-3$

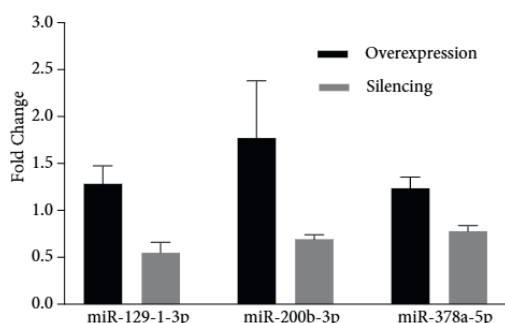


Figure 4. Validation analysis by qRT-PCR of the candidate miRNAs in HNF1A overexpressed and silenced MIN6 cells.

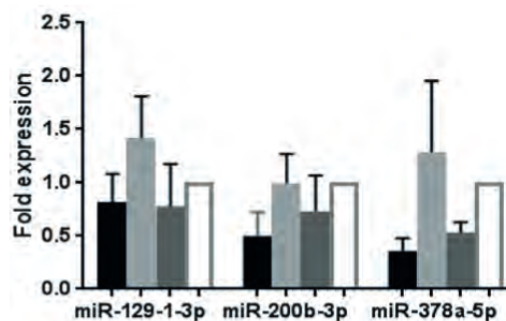


Figure 5. Fold expression changes of the candidate miRNAs in MODY3 patients, type 1 diabetes patients, type 2 diabetes patients, and healthy subjects. Black bars: MODY3 patients (n = 4); light gray bars: type 1 diabetes patients (n = 5); dark gray bars, type 2 diabetes patients (n = 5); white bars: control (n = 3).

Validation analysis by qRT-PCR of the candidate miRNAs in HNF1A overexpressed and silenced MIN6 cells revealed that their expression significantly decreased with the silencing of the HNF1A gene (Figure 4).

The expression level of miR-129-1-3p was decreased in MODY3 and type 2 diabetes patients by 0.8- and 0.7-fold, respectively, and increased by 1.4-fold in type 1 diabetes patients relative to the control group. However, statistical analysis revealed that the decrease in its expression in MODY3 and type 2 diabetes patients was not significantly different ($P > 0.05$), as well as the increase in type 1 diabetes patients (Figure 5).

qRT-PCR analysis of miR-200b-3p in the blood samples of the study populations demonstrated that its expression decreased significantly in MODY3 patients by 0.5-fold relative to the control ($P = 0.011$). In addition, its expression level decreased in type 1 and type 2 diabetes patients compared to the control group, but not significantly ($P > 0.05$) (Figure 5).

Expression analysis of miR-378a-5p in blood samples revealed that its expression significantly decreased by 0.3-fold in MODY3 patients compared to the control group ($P = 0.011$). Comparing its expression in type 2 diabetes patients with controls demonstrated that it was decreased significantly by 0.5-fold ($P = 0.045$). Although its expression increased in type 1 Diabetes patients by 1.28-fold compared to the control group, the difference was not significant ($P > 0.05$) (Figure 5).

4. Discussion

MODY3 associated with the HNF1A gene is the most common form of MODY in adults with 30%–60% prevalence among MODYs.

The HNF1A gene is expressed in the pancreas, liver, and kidneys and encodes for the transcription factor HNF1A. HNF1A regulates expression of the genes involved in glucose metabolism, glucose transportation, and the insulin gene as well as liver-specific genes. HNF4A, for example, is regulated by HNF1A and is required for the normal functioning of the liver and pancreas. It is also a transcription factor that is in a positive feedback mechanism with HNF1A to increase HNF1A expression (16).

There are more than 300 different HNF1A gene mutations in the human genome mutation database. MODY3 exhibits variable clinical phenotypes. The clinical phenotype of MODY3 can vary within the same family even if the patients have the same mutation. Although MODY3 has been associated with HNF1A gene mutations, it presents variable clinical phenotypes even within the same family (17,18). The onset and prognosis of the disease can vary depending on the location of the HNF1A mutation. For example, in patients with mutations between exon 1

and 6, the onset of the disease can be earlier than expected (19,20).

Studies have demonstrated that in addition to the obesity and other genetic loci, the MODY3 phenotype depends on both mutation type and the location of the mutation along the HNF1A gene. MODY3 is frequently misdiagnosed as type 1 Diabetes due to the similar clinical features and the early onset of diabetes. Correct diagnosis of MODY3 is important for preventive treatment since MODY3 patients are under higher risk of diabetic complications such as heart attacks and strokes (21–23). Correct diagnosis of MODY3 is possible by genetic mutation testing. However, despite the cost-effectiveness of direct DNA sequencing analysis, genetic mutation testing is still an expensive and time-consuming process, especially in developing countries.

It is important to determine the molecular mechanism of diabetes, which is considered as an epidemic disease due to increasing prevalence worldwide, to decrease its morbidity and mortality rate. On the other hand, the exact pathogenesis of diabetes has not been resolved yet. Recent studies have revealed that miRNAs have important roles in the development of diabetes, diabetic complications, insulin expression, insulin secretion, and insulin action (24). For example, miR-30d is implicated in insulin gene transcription. Another study demonstrated that miR-103 and miR-224 are involved in MODY3 pathogenesis due to HNF1A Pro29fsinsC mutation in INS-1 insulinoma cell lines (12,14).

miR-375 in pancreatic beta cells has also been shown to be involved in insulin secretion and pancreas development (10,25), miR-133 in cardiac cells has been shown to be involved in diabetic cardiac complications, and miR-192 in the kidneys has been shown to be involved in diabetic nephropathy (26). It has been reported that miR-30d regulates the expression of the MafA transcription factor, which is a protein that inhibits the expression of insulin (27).

In this study, we report the differential expression of miRNAs by overexpression and silencing of the human HNF1A gene in a heterologous cell system. The current report has revealed differential expression of 238 known miRNAs in the miRbase database. Among the 238 identified miRNAs, miR-129-1-3p, miR-129-2-3p, miR-200b-3p, miR-296-3p, miR-378a-5p, miR-324-5p, let-7b-3p, miR-3072-3p, miR-8112, and miR-1983 were the ones having the most significant differential expression by the change in HNF1A expression. miR-3072-3p, miR-8112, and miR-1983 are not present in humans. miR-324-5p, on the other hand, is conserved in both humans and mice. The other 6 miRNAs have been associated with diabetes and/or metabolic pathways related with diabetes in the literature (28).

In this study we also assessed the roles of miR-129-1-3p, miR-200b-3p, and miR-378a-5p as biomarkers to use in the diagnosis of MODY3 patients. miR-129 was shown to exhibit a role in glucose metabolism in earlier studies (29). miR-200b-3p was associated with diabetic nephropathy by inhibiting the expression of FOG2 protein. FOG2 blocks the function of Akt, which is a kinase that induces cell growth. Inhibition of the FOG2 gene by miR-200b prevents its blocking effect on Akt, which can lead to an increase in cell volume in nephrons, therefore causing diabetic nephropathy (30). miR-200b was also reported to facilitate insulin signaling by blocking FOG2 (31). Moreover, overexpression of miR-378a-5p was shown to display hepatic insulin resistance by blocking insulin signaling through targeting p110 α , which is a critical component of insulin signaling (32).

In silico prediction by miRanda and TargetScan of the targets of the selected miRNAs revealed that target genes such as SLC2A glucose transporter family genes, G6PC, IDH2, GPI, and H6PD are involved in glucose metabolism. Functional classification of the target genes in signaling pathways by Panther also predicted target genes in signaling pathways with glucose metabolism. This observation together with literature data imply the role of selected miRNAs in the pathogenesis of MODY3.

Although previous studies have analyzed miRNA profiles and implicated miRNAs in beta cell development and function as well as diabetes pathogenesis, the reflection of these findings on clinical diagnosis has been very limited. MODY has been considered as a rare disease that constitutes 0.6% to 2% of diabetic cases. However, since clinical diagnosis of diabetes has depended on high blood glucose levels, clinical diagnosis of MODY is complicated

due to similar clinical symptoms as type 1 or type 2 diabetes. It is estimated that 80% of MODY cases are misdiagnosed as type 1 or type 2 diabetes (33). Accurate clinical diagnosis of MODY is not only important for proper management of the disease and but also for the reduction of the cost of treatment. With the correct diagnosis, MODY3 is usually treated with sulfonylurea with good results (34), without insulin requirements. However, due to the complexity of clinical diagnosis of MODY, its correct diagnosis is done with genetic diagnosis, which is an expensive diagnosis method. Therefore, availability of biomarkers is important for the accurate diagnosis of MODY.

Earlier studies identified that miR-103 levels are higher in MODY3 patients (12,35). Different from those earlier studies, we observed that expression levels of the selected miRNA molecules decreased in MODY patients significantly, except miR-129-1-3p expression.

In conclusion, although further studies with larger patient numbers are required, the results indicate that miR-200b-3p and miR-378a-5p can be used as biomarkers, which can be isolated from the peripheral blood of suspected patients easily for the diagnosis of MODY3 by selecting patients for HNF1A gene analysis. Our results also indicate that miR-200b-3p is a strong candidate to use for the diagnosis of MODY3 since the expression level of miR-200b-3p was significantly different only between MODY3 and the control group.

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Supplementary Table S1. Total list of the 238 known miRNAs differentially expressed between HNF1A silenced and HNF1A overexpressed.

miRNA name	Normalized expression ratio	P-value
mmu-miR-182-5p	-0.452678799518914	0
mmu-miR-375-3p	0.0638994916997115	0
mmu-miR-741-3p	0.503751734925089	0
mmu-miR-26a-5p	-0.446979195534201	0
mmu-miR-30a-5p	-0.342905490622129	0
mmu-miR-92b-3p	1.19273995425598	0
mmu-miR-99b-5p	0.128683732567218	0
mmu-miR-871-3p	0.129122957214776	0
mmu-miR-434-3p	-0.436202236869665	0
mmu-let-7i-5p	-0.314178822996219	0
mmu-miR-486-5p	0.593818708608856	0
mmu-miR-3107-5p	0.593947138218431	0
mmu-miR-191-5p	-0.177765330389622	0
mmu-let-7a-5p	-0.299038288029997	0
mmu-miR-465a-3p	-0.307350533997947	0
mmu-miR-465b-3p	-0.307318587695004	0
mmu-miR-465c-3p	-0.306777581692746	0
mmu-miR-30c-5p	-0.672878120831967	0
mmu-miR-541-5p	-0.289046362504695	0
mmu-miR-25-3p	-0.358587008501848	0
mmu-miR-129-1-3p	-2.63315184314232	0
mmu-miR-129-2-3p	-2.87047488433413	0
mmu-miR-125a-5p	0.166280400854888	1.69367184355694e-307
mmu-let-7f-5p	-0.106958019665114	3.41919359686734e-266
mmu-miR-470-5p	0.0773382878742092	1.2132940393905e-261
mmu-miR-186-5p	-0.336644361877964	1.25649969747858e-255
mmu-miR-378a-3p	-0.392052811102228	5.53897457519369e-251
mmu-miR-383-5p	-0.621655718998431	3.03274212422102e-241
mmu-miR-148a-3p	-0.907434087186997	2.34042154570019e-220
mmu-miR-30e-5p	-0.269989896907313	1.03884237748353e-213
mmu-miR-30d-5p	-0.196892017106382	2.36177844278362e-211
mmu-miR-22-3p	-0.175677793518122	5.99320613489461e-210
mmu-miR-183-5p	-0.411619502280237	1.28670255035362e-197
mmu-miR-127-3p	-0.0773442183240006	1.38339877932726e-191
mmu-miR-301a-3p	-0.472333966474311	9.14039557470053e-188
mmu-miR-16-5p	-0.240039036278121	9.30816903185997e-184
mmu-miR-141-3p	-0.360265785086673	2.8185483718784e-182
mmu-miR-181a-5p	-0.1110951257089	1.01120647298737e-175
mmu-let-7d-3p	-1.05592176511066	3.51373503338084e-169
mmu-miR-340-5p	-0.194680063418996	2.52285952527764e-133
mmu-miR-879-5p	-1.77985973076281	1.7560185576325e-132

mmu-miR-93-5p	-0.383108641137999	4.67404366029343e-130
mmu-miR-30b-5p	-0.514596520444986	3.41522222847916e-125
mmu-miR-540-3p	0.213066127950805	8.0608689018208e-112
mmu-miR-423-5p	0.184110878318908	2.37707931736613e-111
mmu-miR-27b-3p	-0.089153395670081	7.55345499809308e-105
mmu-miR-181b-5p	0.193469352700873	2.29864921051406e-94
mmu-miR-128-3p	-0.712680110427195	2.66037768821618e-87
mmu-miR-381-3p	-0.221494301024121	1.46990843746614e-85
mmu-miR-431-5p	-0.421256565833643	5.53935149391204e-81
mmu-miR-151-3p	-0.203491318752407	8.21940168086931e-79
mmu-miR-143-3p	0.832946151769782	2.94183883422432e-78
mmu-miR-871-5p	-0.173702877013673	5.35180268527553e-76
mmu-miR-410-3p	-0.26185346288818	1.22058961036415e-74
mmu-miR-465c-5p	-0.14404984059623	1.08546388865468e-71
mmu-miR-872-3p	-0.663269232833981	3.00852565107324e-69
mmu-miR-98-5p	-0.283838002703311	4.16655483549628e-63
mmu-miR-881-3p	0.104001345880619	7.62487412171674e-63
mmu-miR-129-5p	-0.457402695992581	2.95201029301016e-61
mmu-let-7d-5p	-0.222272125975005	9.08971349915732e-61
mmu-miR-100-5p	-1.54165000797003	1.42006042428435e-60
mmu-miR-21a-5p	-0.187692111945951	2.16585084386567e-60
mmu-miR-103-3p	-0.258205160526732	1.97988141926106e-59
mmu-let-7b-5p	0.255172399078458	3.18952868633616e-57
mmu-miR-411-5p	-0.114834089404816	4.15230000627799e-57
mmu-miR-708-3p	-0.712000059778614	1.68353210765087e-51
mmu-miR-30a-3p	-0.289476984628575	1.47420140287068e-48
mmu-miR-30e-3p	-0.221400026953764	4.75667654280608e-48
mmu-miR-106b-3p	-0.344235828666461	3.18904289886218e-46
mmu-miR-1306-5p	-1.88234625970102	1.31642151816964e-44
mmu-miR-15b-5p	-0.447445827582628	1.02297551236049e-42
mmu-miR-409-5p	-0.172135578166912	1.20063807210826e-42
mmu-miR-298-5p	-0.725793535932203	6.06553446238802e-42
mmu-miR-149-5p	-0.632374085625145	4.36470074325946e-41
mmu-miR-192-5p	-0.0770450559628058	4.84100984666721e-41
mmu-miR-342-3p	-0.428480182905717	1.72996132322848e-39
mmu-miR-10a-5p	-0.517509489316492	1.24240924727218e-38
mmu-let-7j	-0.341542514239013	4.7233528623878e-38
mmu-miR-3072-3p	-2.40589693205293	7.73752611907167e-37
mmu-miR-140-3p	-0.318012097399061	1.40826700374423e-35
mmu-miR-29a-3p	-0.228139504957045	3.17397737011653e-35
mmu-miR-8112	-1.91214406363521	4.47945845751826e-35
mmu-miR-484	-0.481051611489073	5.05953528505615e-35
mmu-miR-210-3p	-0.359030105960363	1.23408083849539e-33
mmu-miR-96-5p	-0.459936771765823	9.2531255408457e-33

mmu-miR-339-5p	-1.41762596690786	4.42142599172791e-31
mmu-miR-200b-3p	-1.02156676865635	1.87645565293695e-30
mmu-miR-378a-5p	-2.2402468708092	6.70066742598742e-30
mmu-miR-132-3p	-0.672574625576539	7.18754695234039e-30
mmu-miR-320-3p	-0.305393111675315	1.23738841845537e-29
mmu-miR-421-3p	-0.281221132169808	3.48271266228367e-29
mmu-miR-301b-3p	-0.607388772979687	1.30969991306899e-28
mmu-miR-7a-2-3p	-1.0130972123952	1.24862924159265e-27
mmu-miR-299a-5p	-3.59669490927294	2.760957851923e-27
mmu-miR-324-5p	-2.25525913832853	2.11551828265749e-26
mmu-miR-465b-5p	-0.171275323320362	5.68692139685635e-26
mmu-miR-878-5p	-0.125897022274131	4.11155104460853e-25
mmu-miR-532-5p	-0.271621719311735	2.11018711331628e-24
mmu-miR-341-3p	-0.180022446988417	1.56419190420306e-23
mmu-miR-99a-5p	-0.382198518225657	2.92224023793802e-23
mmu-miR-463-5p	-0.174419055402014	3.18584666914473e-23
mmu-let-7g-5p	-0.13992274926532	3.64310887615647e-23
mmu-miR-126a-5p	-1.10736335943363	1.02568431015465e-22
mmu-miR-6240	-1.78186486665319	1.09339016184513e-22
mmu-miR-377-3p	-0.490119777262201	1.42999519694685e-22
mmu-miR-101a-3p	-0.155560272659514	2.55417988629989e-21
mmu-miR-1839-5p	-0.190566619148516	4.28748529365401e-21
mmu-miR-130a-3p	-0.165193506204693	1.01171012578178e-19
mmu-miR-667-3p	-0.596116570187529	1.38962478759473e-19
mmu-miR-666-5p	-0.359784693573664	2.02993631880947e-19
mmu-miR-136-3p	-0.158095000047297	3.73099515913075e-19
mmu-miR-483-3p	-0.903543423241437	5.50288786133603e-19
mmu-miR-107-3p	-0.250070597727832	6.63023931500389e-19
mmu-miR-344d-3p	-0.690451752922604	1.06627289781449e-18
mmu-miR-379-5p	-1.08975350827124	1.10197980323352e-18
mmu-miR-429-3p	-1.51202528291428	1.24175316642778e-18
mmu-miR-872-5p	-0.239668496007928	2.28786373469554e-18
mmu-miR-743b-3p	-0.0950620672250738	2.76606362542091e-18
mmu-miR-1249-3p	-2.71732461445847	2.54795730303489e-17
mmu-miR-138-5p	-0.301807575523933	4.41695521664073e-17
mmu-miR-1224-5p	-0.247593497063423	5.66875406314911e-17
mmu-miR-325-3p	-0.520740050828024	7.2183076805602e-17
mmu-miR-26b-5p	-0.195262715512335	2.52744460084795e-16
mmu-let-7a-1-3p	-1.1974571419592	3.04236721490505e-16
mmu-let-7c-2-3p	-1.1974571419592	3.04236721490505e-16
mmu-miR-8114	-1.12390714692677	3.31336659331168e-16
mmu-miR-488-5p	-0.54862577847145	5.7205059867273e-16
mmu-miR-666-3p	-0.550221897731884	7.18182638306043e-16
mmu-miR-425-5p	-0.405575685561501	9.69684392910438e-16

mmu-miR-3473b	-2.14443494603789	1.21747667794163e-15
mmu-miR-532-3p	-1.34151627884515	2.87951373859775e-15
mmu-let-7b-3p	-2.45993677176582	7.60187136228245e-15
mmu-miR-184-3p	-0.0948310879899717	1.57449679839132e-14
mmu-miR-200a-3p	-0.836312650675562	3.4157364807605e-14
mmu-miR-378c	-0.503678235586845	3.46261746419981e-14
mmu-miR-369-3p	-0.553494877323201	3.68518195788117e-14
mmu-let-7e-5p	-0.0891740931317544	1.90005866848107e-13
mmu-miR-1983	-2.0057286752063	2.72608366275473e-13
mmu-miR-28a-3p	-0.519599693064162	6.75045649003154e-13
mmu-miR-488-3p	-0.426737428232236	6.82819372770724e-13
mmu-miR-376a-5p	-0.64521602008517	1.63642346437133e-12
mmu-miR-668-3p	-0.512166730538382	3.76772550083002e-12
mmu-miR-3473e	-1.92404487761259	4.80687205292867e-12
mmu-miR-152-3p	-1.13887542069599	5.93709174132294e-12
mmu-miR-574-3p	-1.34513229627502	1.3731431374927e-11
mmu-miR-671-3p	0.242760824510358	1.45684679609256e-11
mmu-miR-328-3p	-0.535180520060232	2.03160021860417e-11
mmu-miR-200c-3p	-0.60827491099999	3.91131029372369e-11
mmu-miR-3078-5p	-0.792561449784689	8.91815939517995e-11
mmu-miR-1981-3p	-1.17466714745731	1.07271617554489e-10
mmu-miR-195a-5p	-0.248629131524952	2.48559061599309e-10
mmu-miR-664-3p	-2.70786428520941	2.67133059147703e-10
mmu-miR-673-5p	-0.189257883184769	2.92314885347336e-10
mmu-miR-183-3p	-1.3264696297582	6.29088758601262e-10
mmu-miR-345-3p	-1.30793367832077	6.37364356559074e-10
mmu-miR-106b-5p	-0.32982463691053	6.88164173636534e-10
mmu-miR-7688-5p	-0.621196912081009	7.79061298825916e-10
mmu-miR-337-5p	-0.237613815968107	8.45754129472629e-10
mmu-miR-126a-3p	-1.60211678272523	1.16519207741709e-09
mmu-miR-199a-3p	-1.13453648497166	2.99119835025869e-09
mmu-miR-199b-3p	-1.13453648497166	2.99119835025869e-09
mmu-miR-329-5p	-0.535167362726721	3.92359590082409e-09
mmu-miR-880-3p	-0.311881844847498	4.71804188861858e-09
mmu-miR-877-5p	0.170847704951424	8.61640035364388e-09
mmu-miR-345-5p	-0.824877755613279	1.15504981638531e-08
mmu-miR-300-3p	-0.19814898559807	1.372577368356e-08
mmu-miR-136-5p	-0.175500320009298	1.78791245607475e-08
mmu-miR-1199-5p	-0.7303886491621	1.96084144860639e-08
mmu-miR-98-3p	-1.05356413361411	2.10251098978552e-08
mmu-miR-1982-3p	-3.54739961301616	3.27943639503821e-08
mmu-miR-540-5p	-1.23252627566275	4.92723687935195e-08
mmu-miR-325-5p	-0.563537013780181	6.75893723466598e-08
mmu-miR-700-5p	-0.921888382381636	8.11518690699869e-08

mmu-miR-7082-3p	-5.74533899062807	8.92533013327541e-08
mmu-miR-743b-5p	-0.263079442536512	1.79292152569821e-07
mmu-miR-331-3p	-2.47116402718908	1.82348440720504e-07
mmu-miR-3068-3p	-0.621617779113228	2.59399108969261e-07
mmu-miR-199a-5p	-5.62986177320814	2.80562668333876e-07
mmu-miR-323-3p	-0.578331472568054	3.95517503037098e-07
mmu-miR-26b-3p	-0.517463948720172	4.1688706330009e-07
mmu-miR-361-3p	-0.381838991494902	6.22163655774215e-07
mmu-miR-153-5p	-0.600546206383581	7.94098778673029e-07
mmu-miR-431-3p	-0.321261209967765	1.23358607692966e-06
mmu-miR-674-3p	-0.495457488117086	1.24224775285115e-06
mmu-miR-487b-3p	-0.350044514848637	1.45903567763177e-06
mmu-miR-1843a-5p	-0.383585885635709	1.50837584983891e-06
mmu-miR-27a-3p	-0.385936190322045	1.65627878513038e-06
mmu-miR-362-3p	-0.786831119546535	2.31562068576395e-06
mmu-miR-574-5p	-0.937984068570467	2.48124993636522e-06
mmu-miR-370-3p	0.21717685032195	3.01801547172665e-06
mmu-miR-382-5p	-0.685715007941653	3.64738428587348e-06
mmu-miR-296-3p	-2.50433089112428	4.13196392781144e-06
mmu-miR-383-3p	-4.36682736737434	5.99751181436721e-06
mmu-miR-758-3p	-0.742538546447116	7.44436572001326e-06
mmu-miR-741-5p	-0.727327582643753	7.88635516720674e-06
mmu-miR-471-5p	-0.473611708662899	9.08215926529089e-06
mmu-miR-124-3p	-0.343137261329243	1.14006775093623e-05
mmu-miR-1981-5p	-0.915616255542013	1.26283854908024e-05
mmu-miR-148b-3p	-0.209675429221931	1.71023039746911e-05
mmu-let-7c-5p	0.0230611386572123	2.32588758897331e-05
mmu-miR-29b-3p	-0.386279730040515	2.40809984569191e-05
mmu-miR-181a-1-3p	0.117621002104981	2.47049260488566e-05
mmu-miR-350-5p	-0.426205914453275	2.47712318330638e-05
mmu-miR-873a-5p	-1.11361202257099	2.59679075765097e-05
mmu-miR-205-5p	4.12502572895533	3.11790271079374e-05
mmu-miR-200b-5p	-5.04489927248698	3.20007791126184e-05
mmu-miR-883b-3p	-0.461167215372084	3.20035883196802e-05
mmu-miR-222-3p	-0.303875461746418	3.22587650434279e-05
mmu-miR-494-3p	-0.506552131040954	3.59587401356126e-05
mmu-miR-451a	0.692066321679227	3.8669103264584e-05
mmu-miR-1191	-0.988315744120612	3.89344819762453e-05
mmu-miR-29c-3p	-0.217787539648586	5.27311667099141e-05
mmu-miR-3057-3p	-2.62986177320814	5.46151704515601e-05
mmu-miR-1839-3p	-1.46939710101489	5.85854108727316e-05
mmu-miR-8103	0.916626579698385	6.14705745790221e-05
mmu-miR-6540-3p	-2.26729169382343	6.30678698672979e-05
mmu-miR-340-3p	-0.1851355826357	7.21946885655249e-05

mmu-miR-194-5p	-0.329749263575639	8.44564172828964e-05
mmu-miR-7679-5p	1.00346374907442	9.13174387633848e-05
mmu-miR-15a-3p	-1.12736143267895	0.000101532892202008
mmu-miR-384-5p	-0.433112983690112	0.000126404496301453
mmu-miR-142-5p	-0.669754500331649	0.00015422257577804
mmu-miR-30c-1-3p	-0.275031954040219	0.000172168658842951
mmu-miR-145a-3p	-3.13236211373732	0.000209334158458209
mmu-miR-742-5p	-1.16828168799226	0.000232005387391383
mmu-miR-7015-3p	0.665594110318035	0.000241198748965684
mmu-miR-146b-5p	-0.119353698124996	0.000252011400236973
mmu-miR-494-5p	-0.413643119491887	0.000263563490600416
mmu-miR-330-5p	-0.353309043199648	0.000331673530564297
mmu-miR-615-3p	-4.62986177320814	0.000382675581585344
mmu-miR-329-3p	-0.749892476914906	0.000387319815339233
mmu-miR-30c-2-3p	-0.134207785737928	0.000404185530480204
mmu-miR-455-5p	-0.354227330594709	0.000441085455532589
mmu-miR-350-3p	-0.786366258888126	0.000454323185739873
mmu-miR-6989-3p	-3.74533899062807	0.000490501477238876
mmu-miR-155-5p	4.54006322823418	0.000496080688496162
mmu-miR-676-5p	-0.57359355356139	0.000665573125754333
mmu-miR-301a-5p	-0.447747362757909	0.000688612809507308